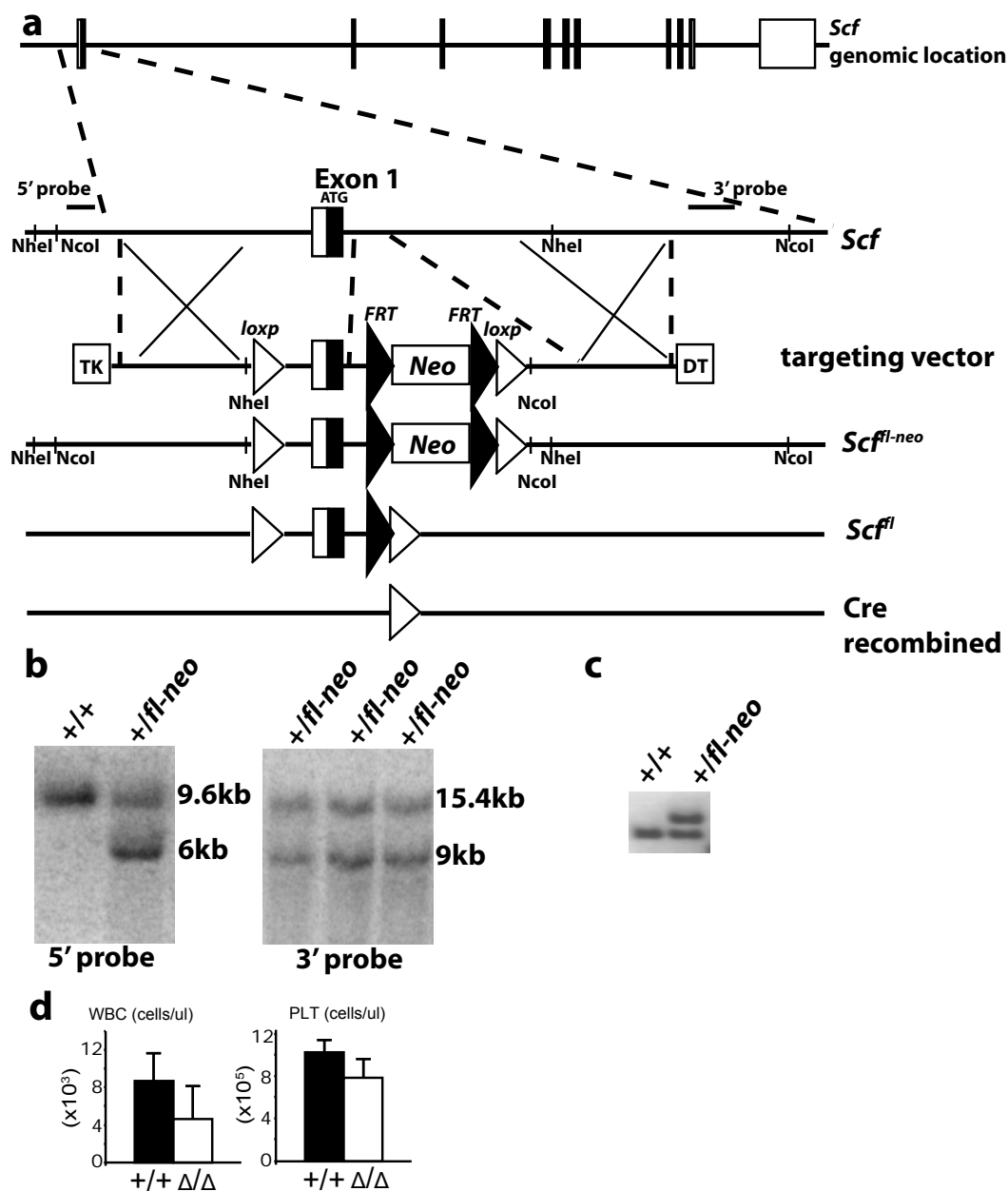Supplementary Figure 1: The generation of *Scf^gfp* mice. a, Targeting strategy to generate the *Scf^gfp* allele. A BAC clone (RP24-339A8) containing the genomic region of *Scf* was used to generate the targeting vector by recombineering. The vector was modified by inserting an *EGFP-polyA-Frt–Neo-Frt* cassette in-frame into the second exon of *Scf.* **b,** The targeting vector was electroporated into W4 ES cells. Correctly-targeted ES clones were identified by Southern blotting using 5' and 3' probes. Chimeric mice were generated by injecting ES clones into blastomeres and were bred with C57BL/6 mice to obtain germline transmission. Then *Scf^gfp-Neo* mice were bred with *Flpe* mice to remove the Neo cassette. These mice were backcrossed at least 5 times onto a C57BL/6 background before analysis. **c,** PCR genotyping demonstrated germline transmission of the *Scf^gfp* allele. **d** and **e,** Heterozygous *Scf^gfp/+* mutant mice exhibited diluted coat color and often a white spot on the abdomen. Arrow in (**e**) points to the white spot on the abdomen. **f**, A typical litter obtained from a *Scf^gfp/+* x *Scf^gfp/+* breeding. Arrows point to two *Scf^gfp/gfp* pups with pale body color. **g,** Progeny generated from mating *Scf^gfp/+* with *Scf^gfp/+* mice. * *Scf^gfp/gfp* pups died perinatally.
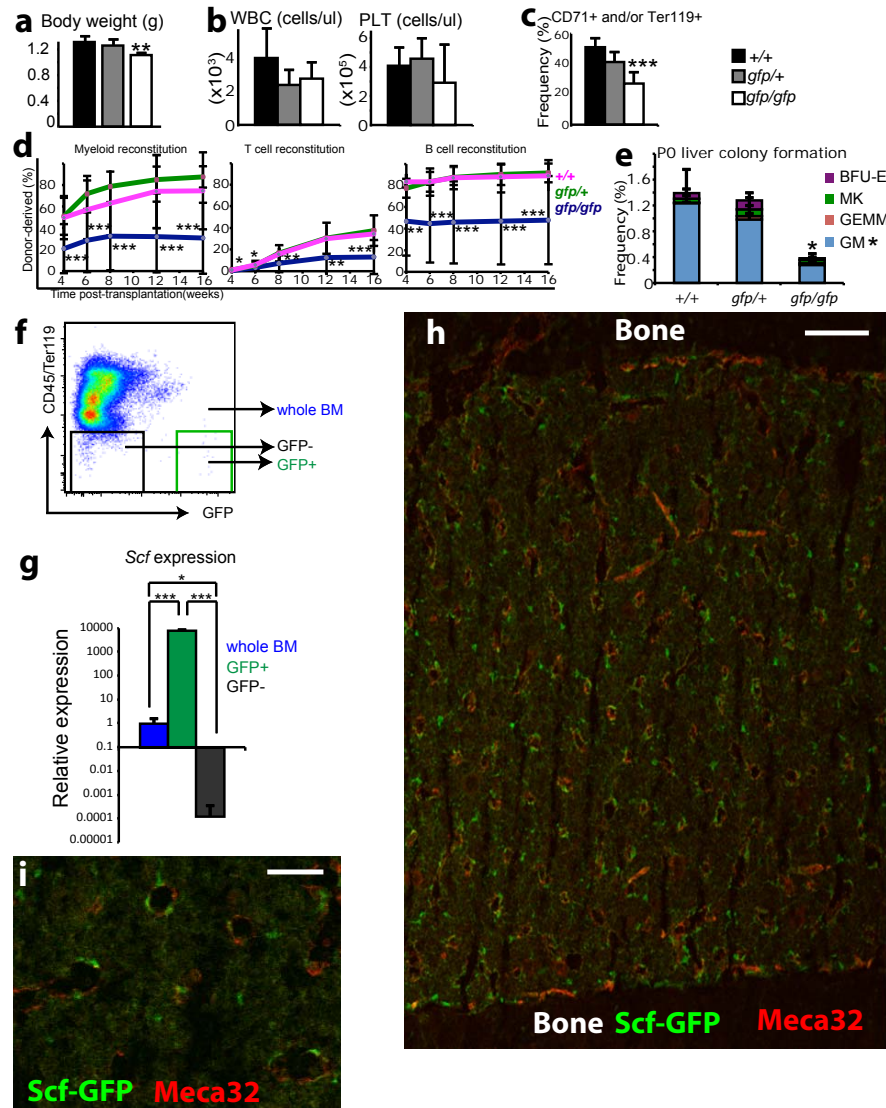
Supplementary Figure 2: *Scf^{gfp/gfp}* **mice exhibit a depletion of HSCs and erythroid progenitors in the bone marrow, and** *Scf* **is primarily expressed by perivascular stromal cells and endothelial cells. a** and **b,** Newborn *Scf^{gfp/gfp}* mice had slightly reduced body weight compared to littermate controls (n=4-9) but exhibited normal white blood cell and platelet counts (n=4-20). **c,** The livers of newborn *Scf^{gfp/gfp}* mice displayed a significant reduction in the frequency of erythroid lineage cells (n=3-4). **d,** Liver cells from newborn *Scf^{gfp/gfp}* mice gave rise to significantly lower levels of donor cell reconstitution in the myeloid, B, and T cell lineages compared to littermate controls. Competitive reconstitution experiments were as described in Fig. 1f (three or four experiments with a total of 13-18 recipient mice per genotype). **e,** Total liver cells from newborn *Scf^{gfp/gfp}* mice gave rise to significantly fewer colonies in methylcellulose compared to *Scf^{gfp/+}* controls (n=4). **f** and **g,** *Scf*-GFP+ stromal cells, *Scf*-GFP negative stromal cells, and whole bone marrow cells were sorted by flow cytometry, and *Scf* expression was assayed by qRT-PCR. *Scf*-GFP+ cells expressed approximately 10,000 fold more *Scf* than whole bone marrow cells, while *Scf*-GFP negative cells expressed at least 10,000 fold less *Scf* than whole bone marrow cells (n=3). **h,** A low magnification image of a bone marrow section from *Scf^{gfp/+}* mice stained with an antibody against GFP (green), and an antibody against Meca32, an endothelial marker (red). Note that *Scf*-GFP was primarily expressed perivascularly throughout the bone marrow. **i,** A high magnification image of a bone marrow section from *Scf^{gfp/+}* mice stained with antibodies against GFP (green) and Meca32 (red). Scale bar is 100um in (**h**) and 20um in (**i**). Data are shown as mean ± s.d.. Two-tailed student's t-tests were used to assess statistical significance. *p≤0.05, **p<0.01, ***p<0.001.

Supplementary Figure 3: The generation of *Scf^fl* mice and characterization of haematopoiesis in *Ubc-CreER; Scf^fl/fl* mice.
a, Targeting strategy. Genomic DNA surrounding *Scf* exon1 was retrieved from BAC (RP24-339A8) by recombineering. The targeting vector was modified by inserting a loxp site 5' of exon1 and an *Frt-Neo-Frt-loxp* cassette 3' of exon1. The insertion sites were carefully chosen so as not to disrupt regulatory elements, by avoiding sequences conserved among species. **b,** The targeting vector was electroporated into Bruce4 G9 ES cells and correctly targeted clones were identified by Southern blotting. **c,** Chimeric mice were bred with C57BL/6-Tyr^c-2J mice to obtain germline transmission (*Scf^fl-neo*). PCR genotyping confirmed germline transmission. *Scf^fl-neo* mice were then bred with *Flpe* mice to remove the Neo cassette and to generate *Scf^fl* mice. **d,** Peripheral blood from *Ubc-CreER; Scf^fl/fl* mice showed lower white blood cell and platelet counts relative to controls, although the differences were not statistically significant (n=5-6). Δ indicates the recombined *Scf^fl* allele, + indicates the wild-type allele. Data are shown as mean ± s.d.. Two-tailed student's t-tests were used to assess statistical significance.
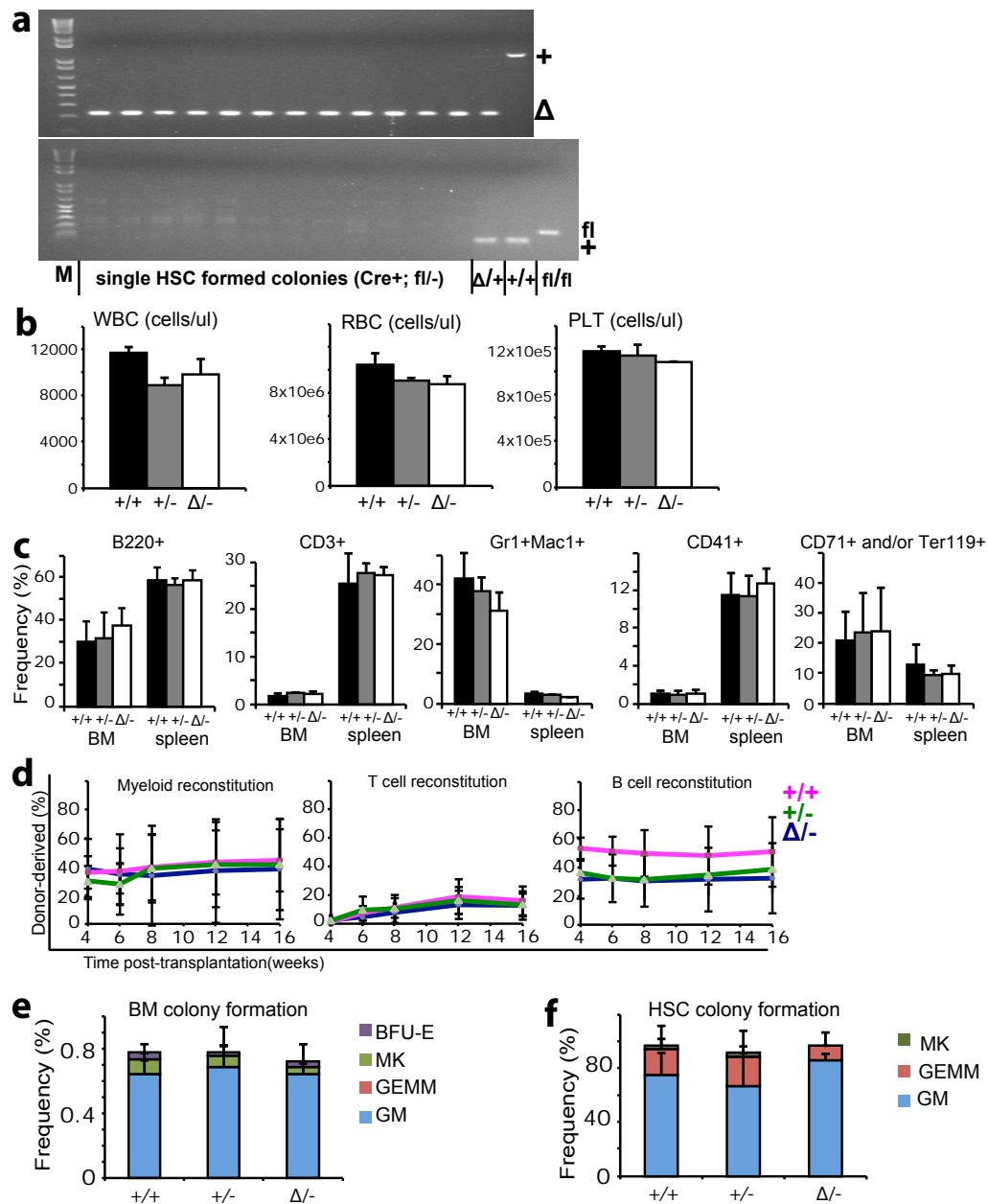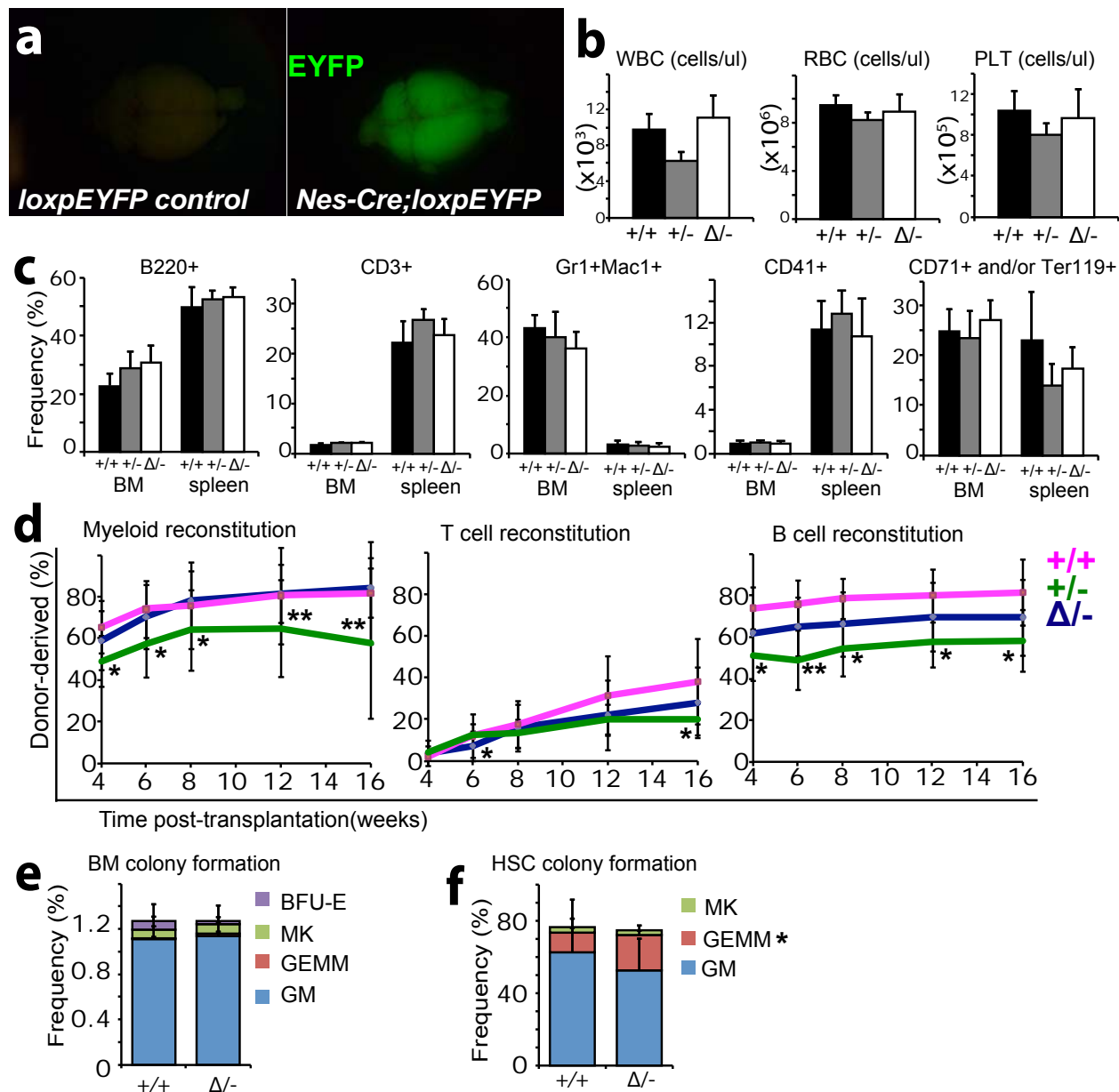
Supplementary Figure 4: *Scf* **deletion from haematopoietic cells in** *Vav1-Cre; Scf*fl/- **mice had no effect on haematopoiesis. a,** *Scf*fl allele recombination in HSCs from *Vav1-Cre; Scf*fl/- mice was complete based on genotyping of colonies formed by individual sorted HSCs. All HSCs (a total of 35/35 from three experiments) had *Scf* recombined. **b,** Peripheral blood from *Vav1-Cre; Scf*fl/- mice did not show significant changes in white blood cell, red blood cell, or platelet counts relative to *Scf*+/- littermate controls (n=4). **c,** Bone marrow and spleen cells from *Vav1-Cre; Scf*fl/- *mice* did not show altered lineage composition (n=4). **d,** Competitive reconstitution experiments described in Fig. 3d showed no difference in the multilineage reconstituting capacity of bone marrow cells from *Vav1-Cre; Scf*fl/- mice compared to littermate controls (two experiments with a total of 10 recipient mice per genotype). **e** and **f,** Bone marrow cells or HSCs from *Vav1-Cre; Scf*fl/- mice exhibited a normal capacity to form haematopoietic colonies in methylcellulose cultures (n=3). Δ indicates the recombined *Scf*fl allele, + indicates the wild-type allele, and – indicates the germline deleted allele of *Scf*. Data are shown as mean ± s.d.. Two-tailed student's t-tests were used to determine statistical significance.
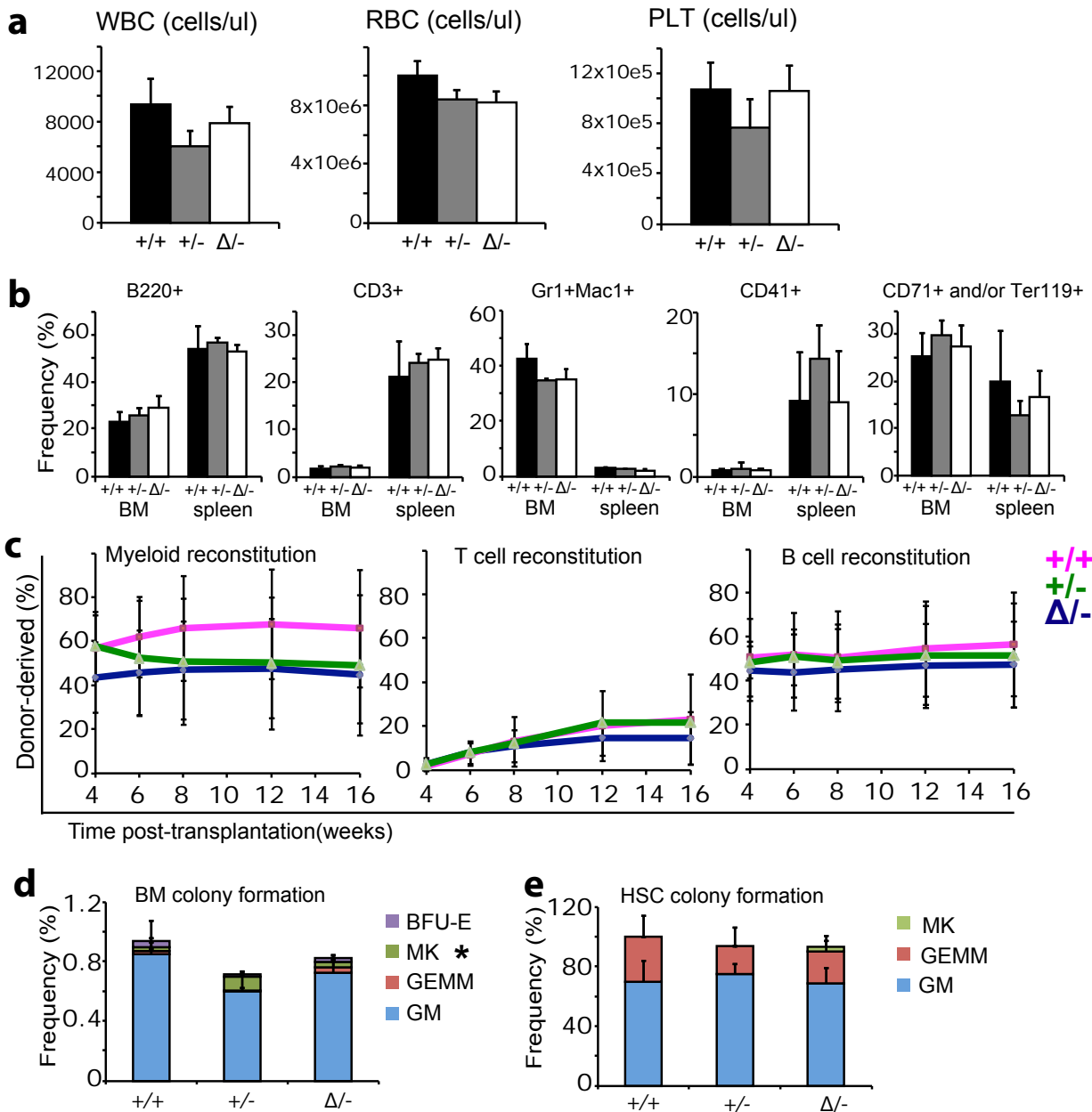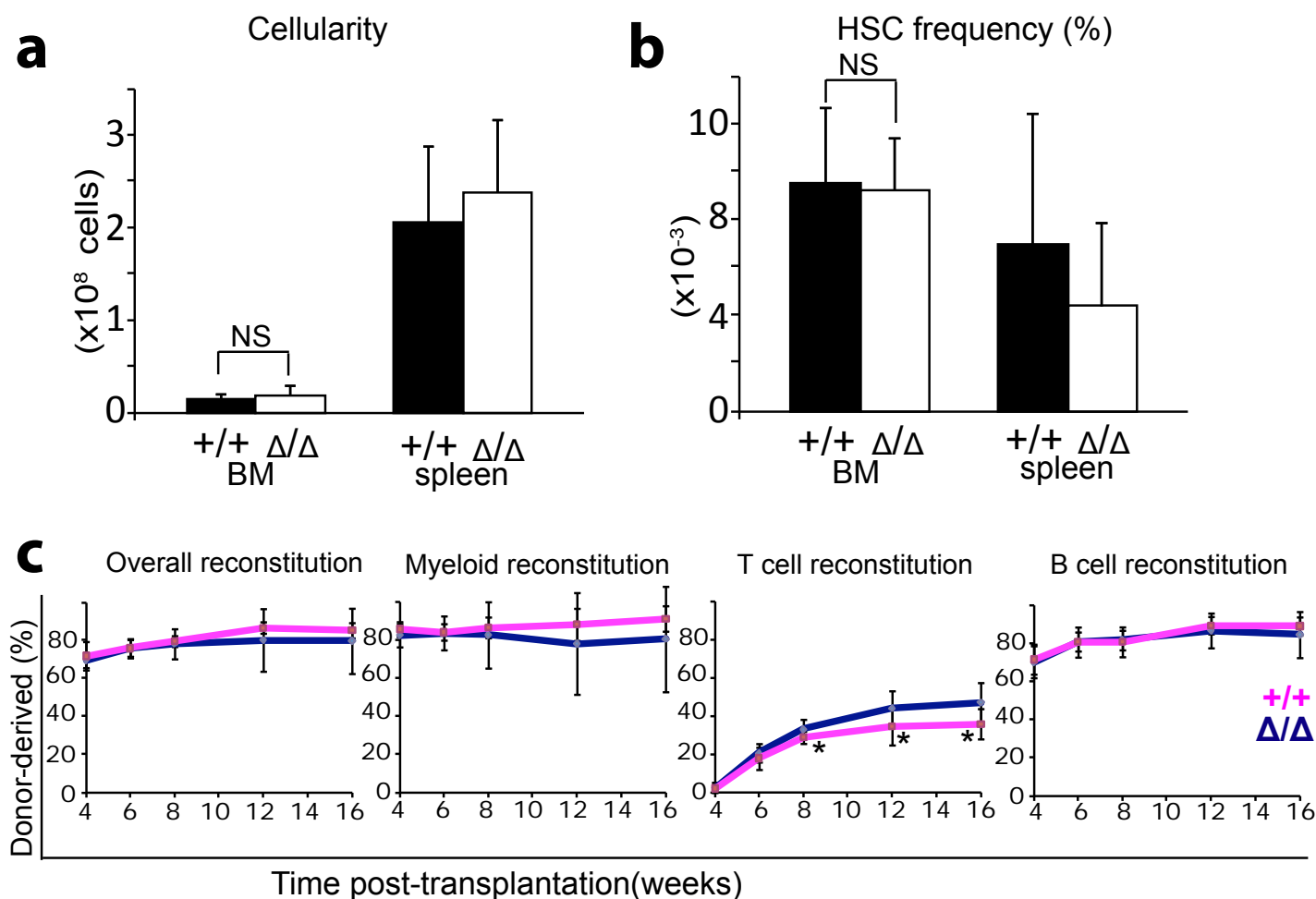
Supplementary Figure 5: *Scf* deletion from osteoblasts in *Col2.3-Cre; Scf^{fl/-}* mice had no effect on haematopoiesis.
**a,** Peripheral blood from *Col2.3-Cre; Scf^{fl/-}* mice did not show significant changes in blood cell counts relative to littermate controls (n=4-5). **b,** Bone marrow and spleen cells from *Col2.3-Cre; Scf^{fl/-}* mice did not show altered lineage composition (n=4-5). **c,** Competitive reconstitution experiments were as described in Fig.3h. Bone marrow cells from *Col2.3-Cre; Scf^{fl/-}* mice did not exhibit significant differences in multilineage reconstituting capacity compared to littermate controls (three to five experiments with a total of 14-22 recipient mice per genotype). **d,** Bone marrow cells from *Col2.3-Cre; Scf^{fl/-}* mice exhibited a significant reduction in megakaryocyte (MK) colony formation compared with *Scf^{+/-}* littermate controls but the frequencies of other colony forming progenitors were normal (n=3-5). **e,** HSCs from *Col2.3-Cre; Scf^{fl/-}* mice did not significantly differ from control HSCs in colony formation capacity (n=3-5). Δ indicates the recombined *Scf^{fl}* allele, + indicates the wild-type allele, and – indicates the germline deleted allele of *Scf*. Data are shown as mean ± s.d.. Two-tailed student's t-tests were used to determine statistical significance. *, p<0.05.

Supplementary Figure 6: *Scf* **deletion from** *Nestin-Cre***-expressing perivascular stromal cells had no effect on haematopoiesis**. **a,** *Nestin-Cre; loxpEYFP* reporter mice displayed broad EYFP expression in the brain, as expected. **b,** Peripheral blood from *Nestin-Cre; Scf*^{fl/-} mice did not show significant changes in blood cell counts relative to littermate controls (n=4-6). **c,** Bone marrow and spleen from *Nestin-Cre; Scf*^{fl/-} mice did not show altered lineage composition (n=5-7). **d,** Competitive reconstitution experiments were as described in Fig. 3l. Bone marrow cells from *Nestin-Cre; Scf*^{fl/-} mice did not exhibit significantly lower reconstituting capacity relative to littermate controls (three to five experiments with a total of 14-24 recipient mice per genotype). **e,** Bone marrow cells from *Nestin-Cre; Scf*^{fl/-} mice exhibited a normal ability to form colonies in methylcellulose (n=3). **f,** HSCs from *Nestin-Cre; Scf*^{fl/-} mice exhibited a significant increase in GEMM (granulocyte, erythroid, macrophage and megakaryocyte colony) colony formation relative to control HSCs but there was no difference in total colony formation (n=3). Δ indicates the recombined *Scf*^{fl} allele, + indicates the wild-type allele, and – indicates the germline deleted allele of *Scf*. Data are shown as mean ± s.d.. Two-tailed student's t-tests were used to determine statistical significance: *p<0.05, **p<0.01.

Supplementary Figure 7: *Scf* deletion from *Nestin-CreER*-expressing cells had no effect on HSC maintenance. a, Deletion of *Scf* from *Nestin-CreER*-expressing cells in *Nestin-CreER; Scf^fl/fl* mice did not significantly affect bone marrow or spleen cellularity relative to littermate controls (n=6-7). **b,** Deletion of *Scf* from *Nestin-CreER*-expressing cells in *Nestin-CreER; Scf^fl/fl* mice did not significantly affect HSC frequency in bone marrow or spleen (n=6-7). **c,** 3x10^5 donor bone marrow cells from *Nestin-CreER; Scf^fl/fl* or *Scf^+/+* mice along with 3x10^5 recipient bone marrow cells were transplanted into irradiated recipient mice. Bone marrow cells from *Nestin-CreER; Scf^fl/fl* mice gave similar or higher levels of donor cell reconstitution in all lineages relative to control cells (two experiments with a total of 9-10 recipients per genotype). Δ indicates the recombined *Scf^fl* allele, + indicates the wild-type allele. Data are shown as mean±s.d. Two-tailed student's t-tests were used to determine statistical significance: *p<0.05. NS, not significant.
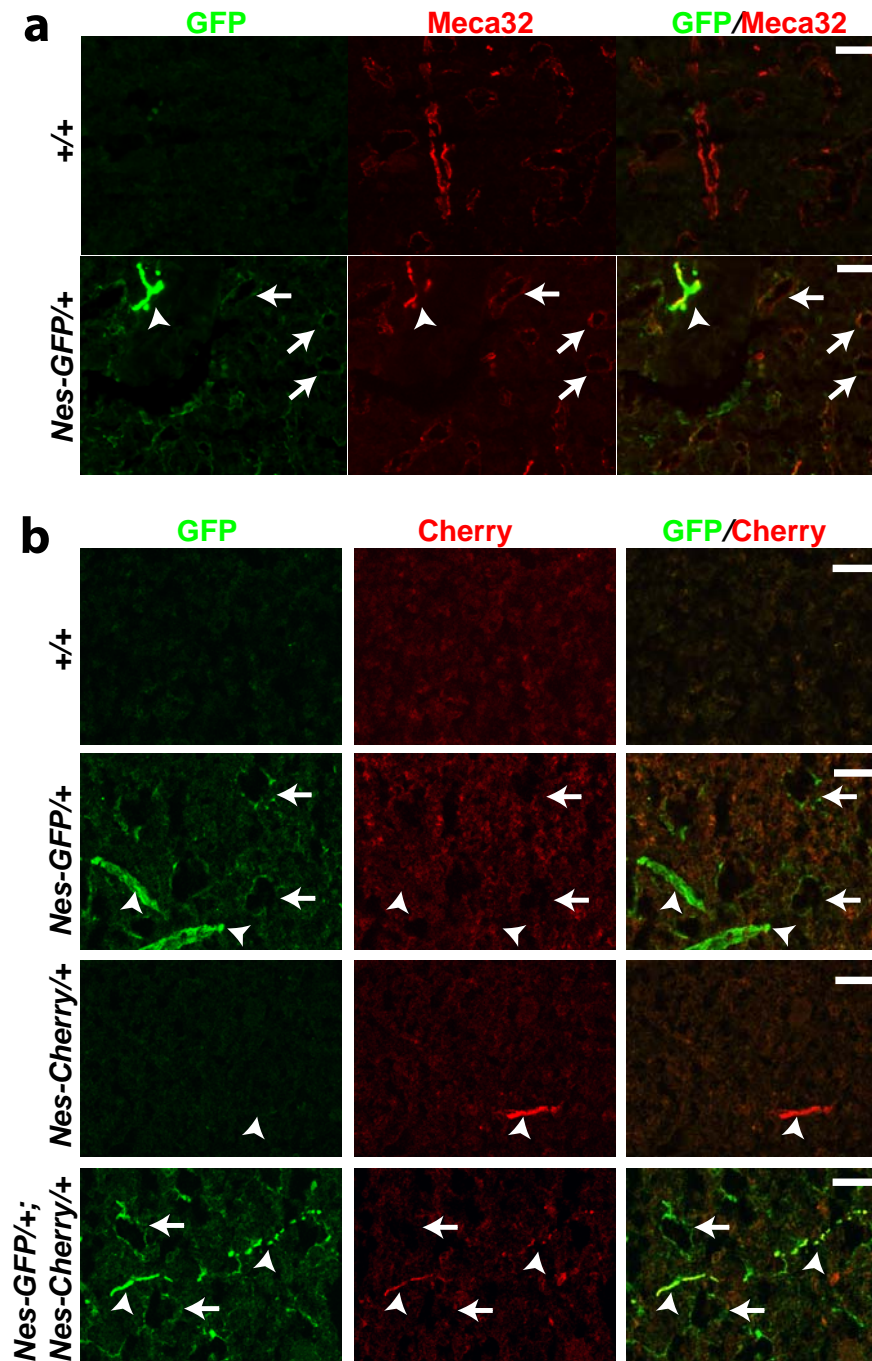
Supplementary Figure 8: *Nestin-GFP* and *Nestin-Cherry* **transgenes exhibit different expression patterns in bone marrow. a,** *Nestin*-GFP (green; anti-GFP antibody) was most strongly expressed by perivascular stromal cells that surrounded blood vessels (arrowheads) in addition to weaker staining by perivascular stromal cells around sinusoids (arrows). Endothelial cells were labeled with anti-Meca32 antibody (red). **b,** *Nestin*-GFP (green; anti-GFP antibody) and *Nestin*-Cherry (red; anti-Cherry antibody) were co-expressed by perivascular stromal cells around larger blood vessels (arrowheads). *Nestin*-GFP was also expressed by perivascular stromal cells around sinusoids while *Nestin*-Cherry was not (arrows). All scale bars are 20um.
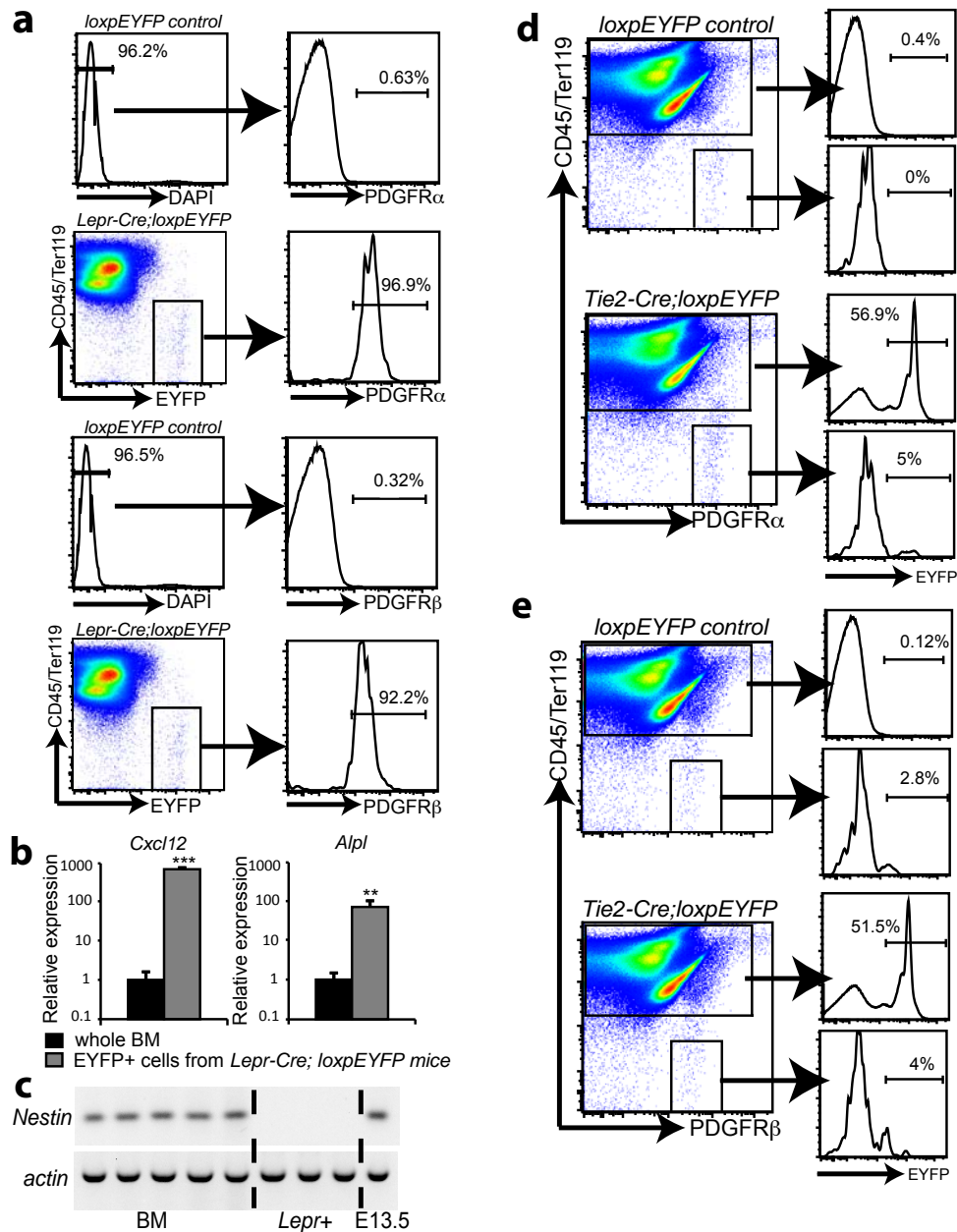
Supplementary Figure 9: *Lepr-Cre* **recombined a conditional reporter in cells that expressed markers of mesenchymal stem/stromal cells but** *Tie2-Cre* **did not. a,** Dissociated bone marrow cells from *Lepr-Cre; loxpEYFP* mice and *loxpEYFP* controls. Almost all the EYFP+ bone marrow cells from *Lepr-Cre; loxpEYFP* mice stained positively for PDGFRα and PDGFRβ, markers of mesenchymal stem cells[33]. **b,** EYFP+ cells isolated by flow cytometry from the bone marrow of *Lepr-Cre; loxpEYFP* mice expressed very high levels of *Cxcl12* and *alkaline phosphatase (Alpl)* by qRT-PCR, genes expressed by mesenchymal stem/stromal cells[15, 16] (n=3). **c,** Endogenous *Nestin* transcript expression was not detectable by RT-PCR in EYFP+ cells from the bone marrow of *Lepr-Cre; loxpEYFP* mice. *Nestin* transcript was readily amplified from whole bone marrow cells and E13.5 whole embryo. **d** and **e,** In contrast to what we observed in bone marrow from *Lepr-Cre; loxpEYFP* mice, mesenchymal stem/stromal cells (CD45/Ter119-PDGFRα+ or CD45/Ter119-PDGFRβ+) from *Tie2-Cre; loxpEYFP* mouse bone marrow were negative for EYFP. Data represent mean±s.d.. Two-tailed student's t-tests were used to assess statistical significance. **p<0.01, ***p<0.001.
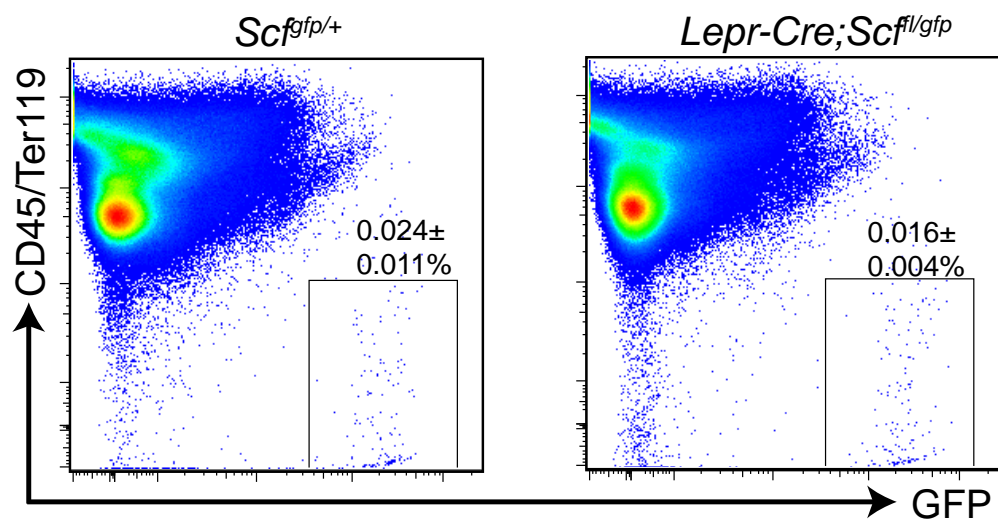
**Supplementary Figure 10: Conditional deletion of *Scf* from perivascular stromal cells did not lead to the depletion of these cells.** The frequency (mean±s.d.) of *Scf*-GFP+ perivascular stromal cells did not significantly differ between the bone marrow of adult *Lepr-Cre; Scf*$^{fl/gfp}$ mice and *Scf*$^{gfp/+}$ controls (n=3-10 mice/genotype). A two-tailed student's t-test was used to assess statistical significance.
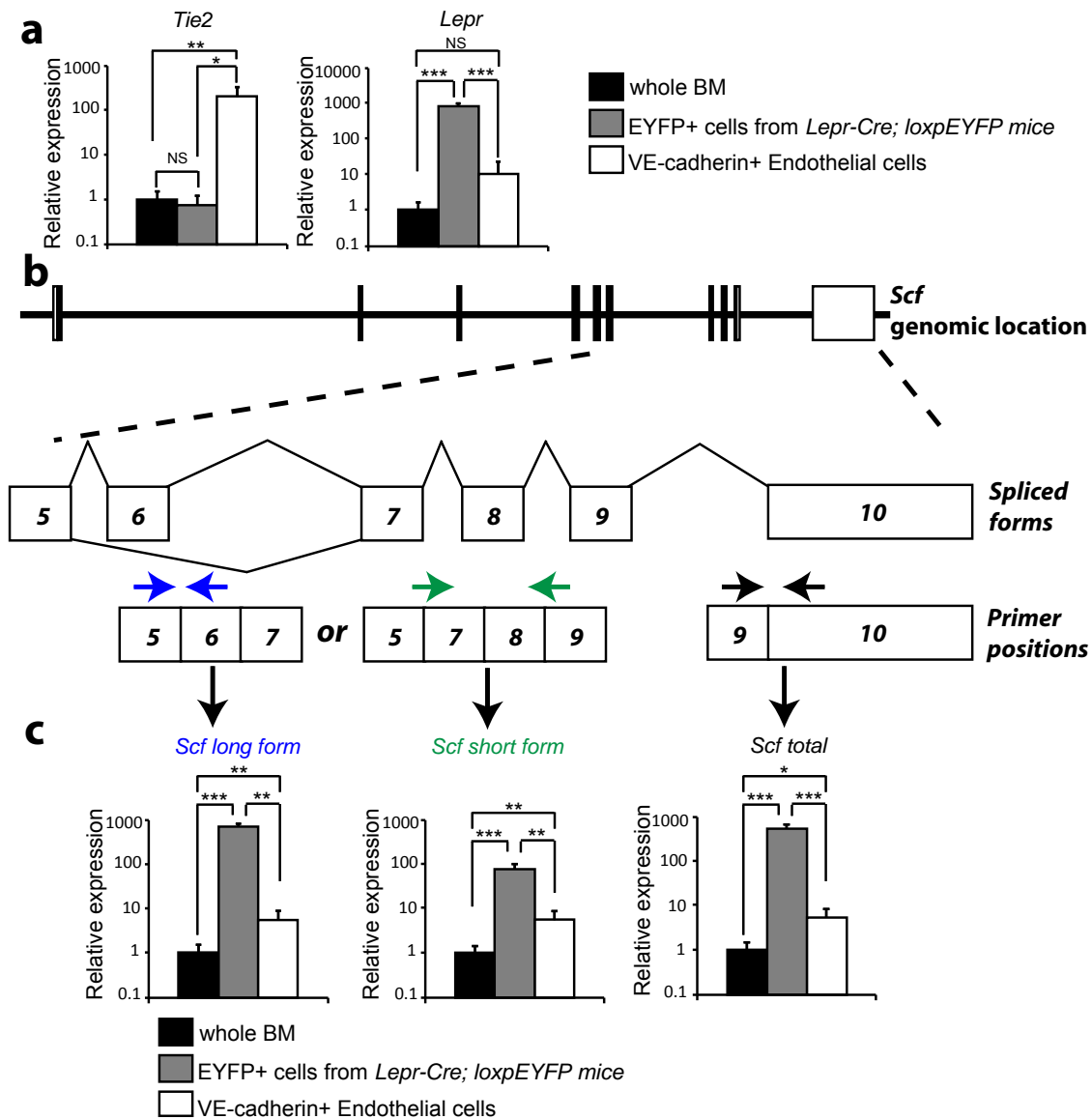
Supplementary Figure 11: Bone marrow endothelial cells and perivascular stromal cells express both splice isoforms of the *Scf* transcript. Each cell type is capable of expressing both membrane-bound and soluble SCF.
a, By qRT-PCR, EYFP+ cells from the bone marrow of *Lepr-Cre; loxpEYFP* mice strongly expressed *Lepr* but not *Tie2*. Conversely, VE-cadherin+ bone marrow endothelial cells strongly expressed *Tie2*, but expressed *Lepr* at levels that were approximately 100-fold lower than EYFP+ cells from the bone marrow of *Lepr-Cre; loxpEYFP* mice (n=3-5). **b,** Schematic illustration of the alternative splicing of *Scf* transcripts and the primers we used to detect each splice isoform. **c,** qRT-PCR revealed that both isoforms of *Scf* (long and short) were expressed by endothelial and perivascular stromal cells at significantly higher levels than detected in whole bone marrow. EYFP+ cells from the bone marrow of *Lepr-Cre; loxpEYFP* mice expressed significantly higher levels of both *Scf* isoforms relative to endothelial cells (n=2-6). Note that in mouse SCF (but not in human SCF) there is a second proteolytic cleavage site in exon 7 that is not alternatively spliced, in addition to the proteolytic cleavage site in exon 6 that is alternatively spliced[51, 52]. So no matter which *Scf* transcript is expressed by a mouse cell, it has the potential to produce both membrane-bound and soluble SCF, depending on the availability of the proteases that can cleave these proteolytic sites. Data represent mean ± s.d.. Two-tailed student's t-tests were used to assess statistical significance. *p≤0.05, **p<0.01, ***p<0.001.
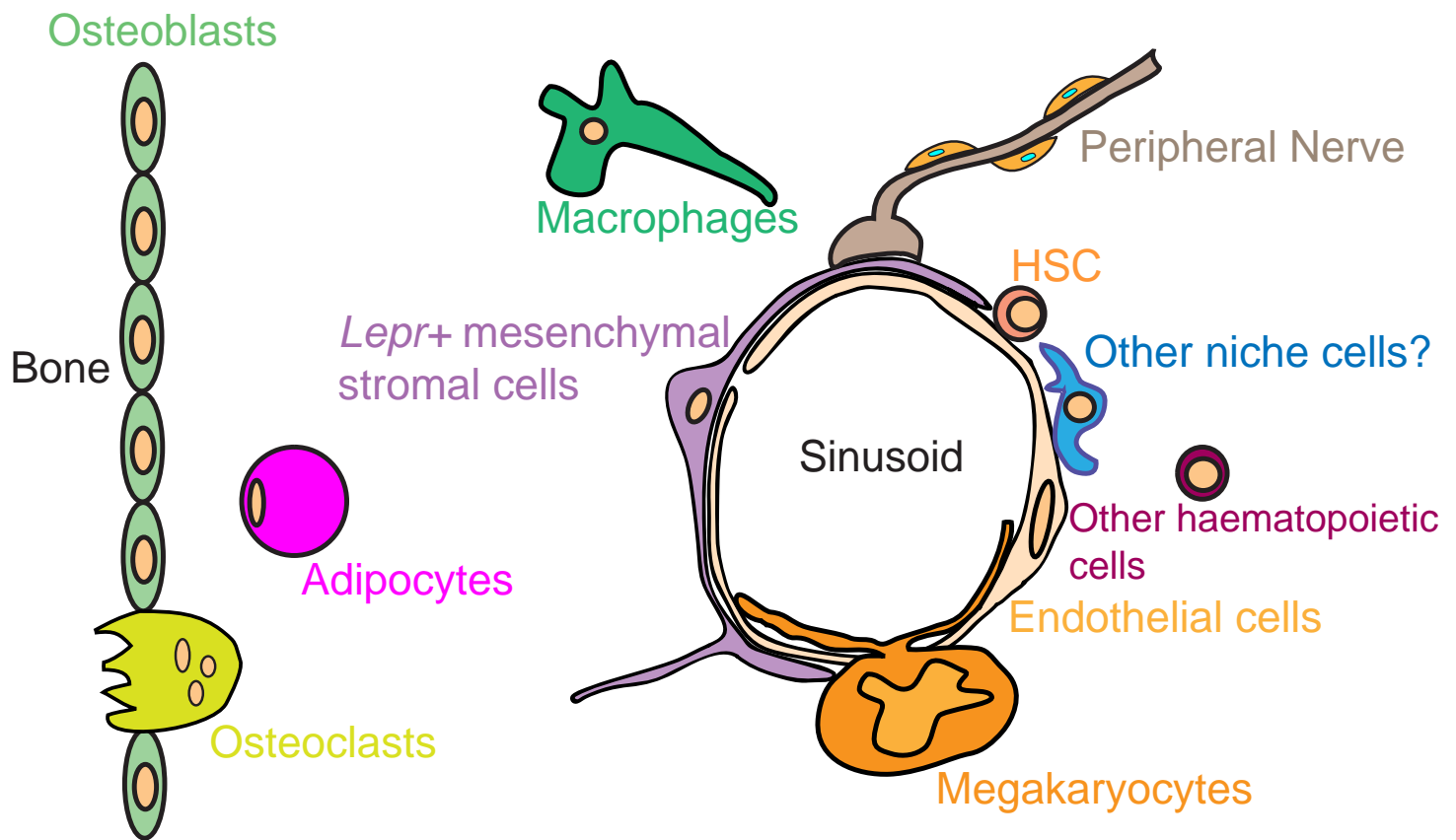
Supplementary Figure 12: A model of the perivascular HSC niche in normal adult bone marrow. HSCs localize primarily around sinusoids in normal adult bone marrow[12-14]. Endothelial cells and *Lepr-Cre*-expressing perivascular stromal cells are two important components of the niche that promote HSC maintenance by expressing SCF and other factors that promote HSC maintenance. The *Lepr-Cre*-expressing perivascular stromal cells likely overlap with other perivascular stromal cell populations that have been implicated in the HSC niche, including *Nestin*-GFP-expressing cells, *Cxcl12*-expressing CAR cells, and perhaps the mouse cells corresponding to CD146+ human stromal cells[14-17]. It is also likely that other perivascular cell populations also contribute to the HSC niche. Other cell populations in the bone marrow that are not necessarily perivascular, such as osteoblasts[39] or macrophages[53-55], may also directly or indirectly regulate the niche, perhaps by secreting factors that act at a distance. Peripheral nerves and glial cells also regulate the HSC niche by innervating perivascular stromal cells[56] and perhaps through other mechanisms[40]. We observed HSCs and *Scf*-expressing perivascular stromal cells throughout the bone marrow (refs[12, 13]; Supplementary Fig. 2h), at sites that were not necessarily close to the endosteum. It remains to be determined whether, and how, this niche changes in response to injury and how its cellular composition differs during development and in extramedullary tissues. Although the *Lepr-Cre*-expressing perivascular stromal cells express markers of mesenchymal stem cells, it is not clear whether these cells include mesenchymal stem cells or whether mesenchymal stem cells are a distinct subpopulation of perivascular stromal cells.

Symble	Description	Scf GFP+	BM	Scf-GFP+/BM	P value
kitl (Scf)	kit ligand	1389.1±375	28.9±4.9	48.1	0.0033
Cxcl12	chemokine (C-X-C motif) ligand 12	8728.3±425.4	133.6±52.1	65.3	4.09805E-06
Nes	nestin	35.7±8.8	30.1±2.8	1.2	0.356516333
Alpl	alkaline phosphatase, liver/bone/kidney	1189.3±218.2	107.4±5.9	11.1	0.001010524
Lepr	leptin receptor	3076.5±537.3	33.9±4.7	90.6	0.000606089
Pdgfrα	platelet derived growth factor receptor, alpha polypeptide	1528.8±124.6	48.6±2.7	31.5	3.30067E-05
Pdgfrβ	platelet derived growth factor receptor, beta polypeptide	3287.1±106	86.5±6.7	38	8.06468E-07
Vcam1	vascular cell adhesion molecule 1	6379.1±146.8	581.1±168.1	11	1.45848E-06
Tek (Tie2)	endothelial-specific receptor tyrosine kinase	86.5±27.4	55.3±10.9	1.6	0.140095411
Plvap (Meca32)	kinase insert domain protein receptor	144.6±7.8	162.9±8.7	0.9	0.052497381
Kdr (Vegfr2)	FMS-like tyrosine kinase 4	74.7±14.5	66.6±15.6	1.1	0.548932611
Flt4 (Vegfr3)	platelet/endothelial cell adhesion molecule 1	58.3±12.6	50.7±8.9	1.2	0.43639413
Pecam1 (CD31)	platelet/endothelial cell adhesion molecule 1	168.4±59.3	604.4±56.4	0.3	0.000767133
Flt1 (Vegfr1)	FMS-like tyrosine kinase 1	136.2±97.2	76.8±9.1	1.8	0.351713698

Supplementary Table 1. The gene expression profile of *Scf*-GFP+ perivascular stromal cells. *Scf*-GFP+ cells were flow cytometrically isolated as in Supplementary Fig. 2f. Several markers of mesenchymal stem/stromal cells were expressed at significantly higher levels in these cells compared to whole bone marrow cells, including *Cxcl12, Alpl, Pdgrfα, Pdgfrβ* and *Vcam1*. These cells also expressed very high levels of *Lepr* relative to bone marrow cells. *Nestin* was not expressed above background. Endothelial cells express much lower levels of *Scf* than perivascular stromal cells (Supplementary Fig. 11) so the gates used in our sorting strategy (which selected cells with high levels of GFP expression, see Supplementary Fig. 2f) preferentially isolated perivascular stromal cells rather than endothelial cells. We did not detect the expression of endothelial cell markers, such as *Tie2, Meca32, CD31, Vegfr1, Vegfr2* and *Vegfr3*, at levels above the background found in whole bone marrow.

Supplementary References

51.   Majumdar, M. K. et al., Identification and mutation of primary and secondary proteolytic cleavage sites in murine stem cell factor cDNA yields biologically active, cell-associated protein. *J Biol Chem* **269** (2), 1237 (1994).

52.   Tajima, Y. et al., Consequences of exclusive expression in vivo of Kit-ligand lacking the major proteolytic cleavage site. *Proc Natl Acad Sci U S A* **95** (20), 11903 (1998).

53.   Chow, A. et al., Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J Exp Med* **208** (2), 261.

54.   Christopher, M. J. et al., Expression of the G-CSF receptor in monocytic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice. *J Exp Med* **208** (2), 251;

55.   Winkler, I. G. et al., Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. *Blood* **116** (23), 4815.

56.   Katayama, Y. et al., Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* **124** (2), 407 (2006).